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Effects of temperature on the development of overwintering pupae of *Pelopidas jansonis* (Butler) (Lepidoptera, Hesperiidae)

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Abstract Overwintering generation larvae of *Pelopidas jansonis* matured and pupated from early October to mid-December with a peak in late October/early November under outdoor conditions in Tsukuba city, Ibaraki prefecture, central Japan. Overwintering pupae were reared under various constant and fluctuating temperatures superimposed on a photoperiod of 12L: 12D (12 h light: 12 h dark) or 15L: 9D. The pupal diapause terminated in January. The photoperiod did not have a significant effect on the duration of the pupal stage, but the thermoperiod had a significant effect on the development of pupae. When pupae were kept under fluctuating temperatures, the pupal period was shorter than that at constant temperatures corresponding to the mean of fluctuating temperatures, especially under lower temperature conditions. The developmental zero and the thermal constant of pupae were 13.0–13.3°C and 158–165 day-degrees and 12.2–12.3°C and 171–173 day-degrees, when pupae were reared under constant and fluctuating temperature conditions, respectively. Pupae may develop faster in the field by using this mechanism in early spring when the surrounding temperature is low.

Key words Developmental zero, diapause termination, grassland butterfly, photoperiod, pupation, seasonal adaptation, thermal constant, thermoperiod.

Introduction

Pelopidas jansonis (Butler) is distributed in Korea, Northeast China and Japan (Honshu, Shikoku and Kyushu). In most of the Japanese localities, this species is bivoltine, with hibernation occurring during the pupal stage (Fukuda *et al*., 1984*b*; Shirôzu, 2006; Chiba, 2007). In Japan, *P. jansonis* is generally regarded as a rare species (Shirôzu, 2006; Chiba, 2007), and has been declining in recent years (The Japanese Society of Environmental Entomology and Zoology, 1998; Inoue, 2005). *P. jansonis* is recognized as an endangered species in some prefectures of Japan (Sunose and Eda, 2003), although the species is not listed in the National Red Data List (Ministry of the Environment Japan, 2007).

Identifying the physiological characteristics of an insect species may offer us basic data to understand the causes of decline, which would be particularly useful if the conservation of the species is needed in the future. Although the life cycles in the wild have been clarified for almost every Japanese butterfly species (Fukuda *et al*., 1982, 1983, 1984*a*, *b*), the fundamental physiological characters such as developmental zero and thermal constant are known for only a few species (Kiritani, 1997). The present study mainly reports on the time of diapause termination and the parameters of the law of total effective temperature (developmental zero and thermal constant) in overwintering pupae of *P. jansonis*. Based on the data, the importance of thermoperiod to adult emergence in the spring is discussed.

Materials and methods

Insects

Larvae living in tube-like shelters (Fukuda *et al*., 1984*b*) made of *Miscanthus sinensis*

(Gramineae) leaves, one of the major larval food plants, were collected from fields in the southern part of Ibaraki Prefecture, central Japan in October–November of 2006 and 2007. They were reared individually under quasi-natural conditions; *i. e.* they were kept in transparent cups (125 × 125 mm at the base, 57 mm height) which were placed in a wire-mesh cage (4 m^2 × 2.5 m in height) in the field of the Forestry and Forest Products Research Institute (FFPRI), Tsukuba City, Ibaraki Prefecture (36°00′N, 140°08′E, 25 m a.s.l.). *M. sinensis* leaves were provided as food. The development of larvae was checked every 2–3 days until they pupated. Pupae were kept in the same cage until the start of experiments conducted in winter.

Experimental conditions

Pupae were reared at constant (17, 20, 23, 25 or 28°C) or fluctuating (cryophase/thermophase=14/20, 17/23, 20/26, 22/28, 25/31 or 17/29°C) temperatures superimposed on a photoperiod of 12L: 12D (short day condition; 12 h light: 12 h dark) or 15L: 9D (long day condition). The fluctuating temperature was composed of 12 h thermopase and 12 h cryophase. The midpoint of thermophase coincided with the midpoint of photophase. The emergence of adults was checked every day and the duration of the pupal period was determined.

Time of diapause termination

To determine the time of diapause termination under natural conditions, overwintering pupae were transferred from the outdoors to an incubator maintained at a mean temperature of 20 (17/23) °C under either a short or long day condition. Transfers were conducted seven times from 26 December 2006 through 6 March 2007.

Developmental zero and thermal constant

Incubation was started on 28 January 2008. Pupae were kept at 11 different constant and fluctuating temperature regimes under the short day condition (Table 3). The developmental zero and the thermal constant were estimated by the conventional equation and the equation proposed by Ikemoto and Takai (2000, 2001).

Conventional equation: $1/D = -(t/k) + (1/k)\,T,\ k = 1/\beta,\ t = \alpha/\beta$
Ikemoto-Takai equation: $(DT) = k + tD,\ k = \alpha,\ t = \beta$

where D, T, t and k represent the duration of development (days), environmental (mean) temperature (°C), the estimated developmental zero temperature and the thermal constant, respectively. Also, β and α represent the slope and intercept of the regression line, respectively.

Results

Time of pupation under quasi-natural conditions in autumn

Pupae of *P. jansonis* are covered with a white waxy powder (Fukuda *et al.* 1984*b*). In the present study, prepupae were also covered with the similar powder; thus larval maturation was judged by the presence of the powder. Larvae matured and pupated from early October to mid-December with a peak in late October/early November (Fig. 1). The duration of the prepupal period (larval maturation to pupation) increased as the date of larval maturation was delayed (Table 1). When larvae matured in October, the mean duration of the prepupal period was less than 5 days. The last pupation occurred in mid-December after a long prepupal period (more than 20 days). Eight larvae matured in or after late November, but they

Table 1. Duration (d) of the prepupal period under a quasi-natural condition. Three hundred seventy pupae obtained in 2007 were used for the calculations.

Date of maturation	Range	Mean	Variance	No. of pupae tested
before Oct. 20	2–7	4.5	5.1	6
Oct. 21–31	2–9	4.7	2.7	155
Nov. 1–10	2–10	6.3	1.9	145
Nov. 11–15	4–19	9.4	13.5	52
Nov. 16–20	12–26	19.8	23.2	12

Table 2. Performance of overwintering pupae transferred from outdoor conditions to an incubator regulated at a mean temperature of 20°C (Cryophase/Thermophase=17/23°C), from December 2006 through March 2007.

Date of transfer	12L: 12D					15L: 9D					Difference between the two photo-periods [‡]
	No. of pupae examined	Rate of adult emergence (%)	Duration of pupal period under incubation			No. of pupae examined	Rate of adult emergence (%)	Duration of pupal period under incubation			
			Range	Mean[†]	Variance			Range	Mean[†]	Variance	
Dec. 26	8	63	27–45	36.4[a]	63.8	8	63	29–61	40.6[a]	171.3	NS
Jan. 15	7	86	21–27	23.8[b]	4.6	7	86	22–34	24.7[b]	21.5	NS
Jan. 25	7	86	20–25	22.0[b,c]	3.2	8	100	21–35	24.3[b]	20.5	NS
Feb. 4	8	88	18–21	19.6[b,c]	1.6	7	86	20–22	20.7[b]	0.7	NS
Feb. 14	10	90	17–22	19.8[b,c]	3.7	10	100	18–22	19.7[b]	1.8	NS
Feb. 24	10	90	16–20	18.2[c]	1.9	10	90	18–30	21.4[b]	11.8	*
Mar. 6	10	90	16–20	17.7[c]	2.3	10	80	16–20	17.9[b]	2.1	NS

[†] Means followed by the same letter among the same photoperiod are not significantly different (p>0.05; by Tukey's HSD test).

[‡] *: significantly different by t-test (p<0.05).

Table 3. Pupal period under various constant and fluctuating temperatures. Overwintering pupae were transferred from the outdoors to an incubator on 28 January 2008 and kept under a photoperiod of 12L: 12D.

Experiment group	Temperature (°C)		No. of individuals examined	Duration of pupal period under incubation		
	Cryophase	Thermophase		Range	Mean	Variance
C17	17		20	34–56	39.4	38.8
C20	20		26	20–30	24.9	8.7
C23	23		23	13–21	17.0	5.4
C25	25		25	11–15	13.3	1.1
C27	27		25	10–15	11.4	1.8
F17	14	20	19	27–45	35.9	19.5
F20	17	23	18	17–26	21.6	6.7
F23A	20	26	23	13–33	17.1	17.9
F23B	17	29	25	13–18	15.6	2.1
F25	22	28	21	11–16	13.3	1.6
F27	24	30	27	10–14	11.5	1.2

Means were significantly different between C20 and F20 and between C23 and F23B (p<0.05; by t-test).

Table 4. Developmental zeros (t) and the total effective temperatures (k) for emergence as adult in overwintering pupa of *P. jansonis* after diapause termination estimated by the conventional and Ikemoto-Takai methods.

	Conventional method		Ikemoto-Takai method	
	t (°C)	k (day-degrees)	t (°C)	k (day-degrees)
Constant temperature	13.34	157.57	12.97	164.79
Fluctuating temperature†	12.31	170.96	12.20	172.95

†The results of F23B (Table 3) were excluded from the calculation.

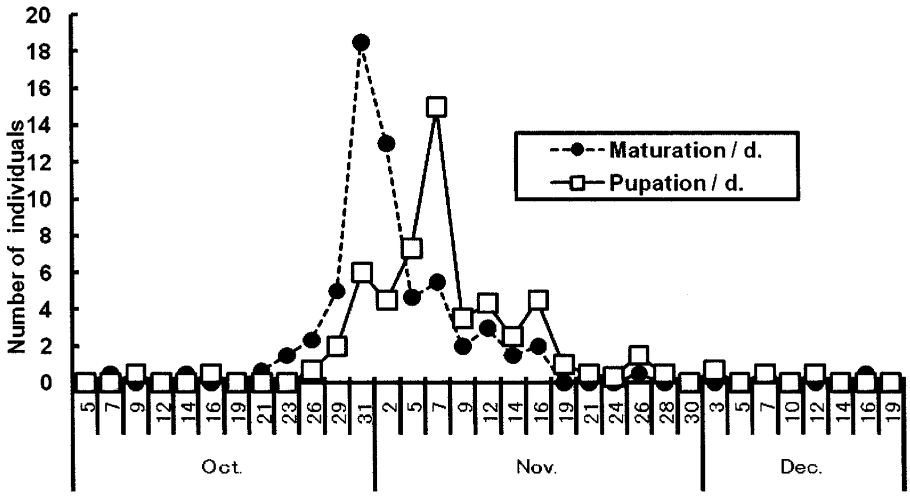

Fig. 1. Times of larval maturation and pupation of *Pelopidas jansonis* under quasi-natural conditions in 2007 based on 147 larvae collected on 3 October 2007 at one locality of Ushiku-shi, Ibaraki Prefecture.

died before pupation.

Time of diapause termination

When the transfer was conducted in late December, the rate of adult emergence was relatively low (63%) and the mean incubation time to adult emergence (duration of the pupal period) was more than 30 days under both short and long day conditions (Table 2). After mid-January, the rate of successful emergence rose to 80% or more. The duration of the pupal period was shorter for later start of incubation. Under short day conditions, when the transfer was conducted between late January and early March, the duration of the pupal period was approximately 20 days and means did not differ significantly among the five groups (by Tukey's HSD test, p>0.05). Under long day conditions, when the transfer was conducted between mid-January and early March, the means did not differ significantly among the six groups. The mean duration of the pupal period under long day conditions was slightly longer than that under short day conditions on most occasions, but the means under the two photo regimes did not differ significantly from each other except in one case (when the transfer was conducted in late February).

Effect of fluctuating temperature on pupal development

The mean durations of the pupal period of the F20 and F17 groups were 3.3 and 3.5 days shorter than those of the C20 and C17 groups, respectively (Table 3). The mean of F20 was significantly different from that of C20 (p<0.01, by t-test), but the difference between the F17 and C17 was only marginally significant (p=0.051). The mean of the F23A group

(temperature range was 6°C) was nearly equal to that of C23, but that of F23B (temperature range was 12°C) was significantly different from that of C23 ($p<0.05$). The means of F25 and F27 were nearly equal to those of C25 and C27, respectively.

Developmental zero and thermal constant

The developmental zero and the thermal constant were 13.0–13.3°C and 158–165 day-degrees and 12.2–12.3°C and 171–173 day-degrees, when pupae were reared under constant and fluctuating temperature conditions, respectively (Table 4). There were few differences between parameters estimated by the conventional equation and those estimated by the Ikemoto-Takai equation, especially when data obtained from the fluctuating temperature condition were used for the calculation.

Discussion

The present study showed that the pupation of the overwintering generation of *P. jansonis* takes place mainly in late October to early November in the lowlands of the Kanto region in central Japan. A similar result has been obtained in Tochigi, the adjacent prefecture to Ibaraki (Kuzuya, 2000). Overwintering pupae are in a state of diapause. As diapause proceeds, insects usually show a progressive loss in their sensitivity to photoperiod, and by mid-winter, photoperiodic sensitivity has ceased in many species (Tauber and Tauber, 1976). Although the photoperiodic condition did not affect the development of overwintering pupae of *P. jansonis* in any season, the diapause seems to have terminated by mid-winter (late January) in the field because the rate of successful adult emergence became higher and the duration of the pupal period did not differ significantly after this season under either short or long day conditions.

Beck (1983) showed that developmental responses to thermoperiods (daily cycles of temperature) vary among different insect species. In some cases, developmental periods are shortened by thermoperiods (*e. g.* Yamashiro *et al.*, 1998). For example, in butterflies, the developmental period (egg to adult emergence) of *Narathura bazalus* (Lycaenidae) reared under fluctuating temperature was significantly shorter than that under constant temperatures, especially when the rearing temperatures were relatively low (Aso *et al.*, 2006). Bryant *et al.* (1999) showed that the development under fluctuating temperatures (cryophase/thermophase=10/20°C) was faster than at a constant temperature of 15°C in four species of Nymphalidae.

In the present study, the fluctuating temperature also shortened the pupal period of *P. jansonis* in some experimental groups. As the mean temperature decreased, the accelerating effect of fluctuating temperature became more conspicuous. Moreover, under a mean temperature of 23°C, a wide range of diurnal temperatures hastened the development.

In most butterfly species, the developmental zeros are around 10°C (Kiritani, 1997; Kato, 2005), although there are some exceptions (*e. g.* Komeyama and Hoshikawa, 2007). The developmental zero of *P. jansonis* pupae estimated by the conventional and Ikemoto-Takai methods was 12–13°C and this is slightly higher than those in pupae of other Japanese butterfly species.

P. jansonis lives in sunny grasslands and pupae sometimes pass the winter near the ground (Fukuda *et al.*, 1984*b*). At overwintering sites that are exposed to the direct rays of the sun, the range of diurnal temperatures may be very wide during winter and spring (Inoue, 2008). The accelerating effect of the fluctuating temperatures may enable pupae to develop rapidly after diapause termination. As a result, adults of *P. jansonis* may be able to emerge early in

the spring, although the developmental zero is relatively high.

Most grassland species of Hesperiidae, *e. g. Pyrgus maculatus*, *Leptalina unicolor*, *Potanthus flavum* and *Polytremis pellucida*, usually become multivoltine in warmer regions but univoltine in northern or mountainous habitats (Fukuda *et al*., 1984*b*). However, *P. jansonis* can produce two generations per year even in Aomori, the northern limit of its geographical distribution (Fukuda *et al*., 1984*b*; Shirôzu, 2006). Further studies are needed to reveal the regulation mechanism of the life cycle in *P. jansonis*, especially the larval response to photoperiod. Also, the effects of temperature on the development of more species of butterflies in relation to their overwintering sites must be studied.

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摘　　要

ミヤマチャバネセセリ越冬蛹の発育におよぼす温度の影響 (井上大成)

ミヤマチャバネセセリの越冬世代幼虫の老熟・蛹化は, 茨城県つくば市の野外条件で10月上旬から12月中旬に起こり, そのピークは10月下旬から11月上旬であった. 幼虫が10月に老熟した場合には前蛹期間は平均約5日だったが, 11月半ばを過ぎて老熟した場合には前蛹期間は平均約20日になった. 越冬蛹が, 12L: 12D または 15L: 9D の日長条件下の様々な温度で飼育された. 蛹休眠は野外条件で1月に覚醒した. 日長条件は蛹期間の長さにほとんど影響しなかったが, 温度の日変化は蛹の発育に影響を与えた. 変温条件で蛹が飼育された場合, 平均温度が同じ恒温条件よりも, 特に低温の場合に蛹期間が短くなった. 越冬蛹の発育零点と有効積算温度は, 恒温条件では13.0–13.3°C と 158–165 日度, 変温条件では12.2–12.3°C と 171–173 日度で, 変温条件で発育零点が低かった. 蛹は周囲の温度が低い早春に, このメカニズムを使って早く羽化することができるだろう.

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